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REVIEW OF THE RECENT LITERATURE ON THE HEALTH ASPECTS OF VITAMIN B_{12} AS A FOOD INGREDIENT

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Prepared for

BUREAU OF FOODS
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by

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FOREWORD

The Life Sciences Research Office (LSRO), Federation of American Societies for Experimental Biology (FASEB) provides scientific assessments of topics in the biomedical sciences. Reports are based upon comprehensive literature reviews and the scientific opinions of knowledgeable investigators engaged in work in specific areas of biology and medicine.

This technical report was prepared for the LSRO Select Committee on GRAS Substances (SCOGS) as a part of their review of the health aspects of using these food ingredients as stipulated in the Food, Drug, and Cosmetic Act for Generally Recognized as Safe substances. Dr. Michael J. Wade prepared the report based on a comprehensive search and evaluative assessment of the current literature in accordance with the provisions of contract no. FDA 223-75-2004. Acknowledgment is made of the assistance of the LSRO staff who provided much of the background information.

Kenneth D. Fisher, Ph.D.
Director
Life Sciences Research Office

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INTRODUCTION

This report concerns the health aspects of using vitamin B_{12} as a food additive. It reviews the world scientific literature published from 1971 through 1976.

To assure completeness and currency as of the date of this report, information has been obtained by searches of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; and by the combined knowledge and experience of members of the LSRO staff. This report supplements and updates information contained in a scientific literature review (monograph) prepared for FDA by Tracor Jitco Inc. (1974)[1].

Vitamin B_{12} is listed in the Code of Federal Regulations[2] (21 CFR 182.5945) as a nutrient and/or dietary supplement. The vitamin was originally isolated as cyanocobalamin but there are several forms. The basic structure of vitamin B_{12} is depicted in Figure I. The various forms differ only in the nature of the R group covalently bound to the cobalt atom. Cyanocobalamin occurs only in trace amounts in the body. Most of the vitamin B_{12} in the body is present as hydroxocobalamin, methylcobalamin or deoxyadenosylcobalamin (Matthews, 1974). Methylcobalamin and deoxyadenosylcobalamin are the coenzyme forms of the vitamin; hydroxocobalamin and deoxyadenosylcobalamin are thought to be the forms present in food.

Oral or intramuscular cyanocobalamin is the treatment of choice for dietary vitamin B_{12} deficiency (Herbert, 1975). Deoxyadenosylcobalamin or hydroxocobalamin have no therapeutic advantage over cyanocobalamin.

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[1] The document is available from the National Technical Information Service, U. S. Department of Commerce, P. O. Box 1553, Springfield, Virginia 22161.

[2] Office of the Federal Register, General Services Administration. 1977. Food and Drug Administration: rules and regulations. Food for human consumption: reorganization and republication. Fed. Regist. 42:14301-14469.

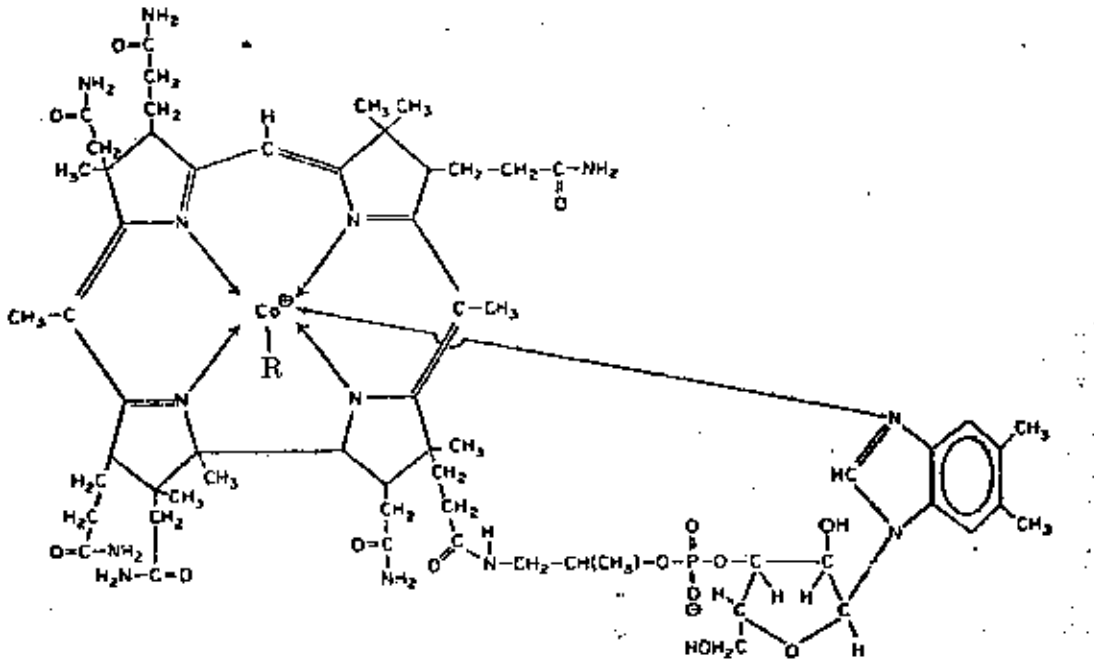

Figure I. Structure of Vitamin B_{12}

R = cyano function for cyanocobalamin; hydroxy function for hydroxocobalamin, methyl function for methylcobalamin and deoxyadenosyl function for deoxyadenosylcobalamin.

I. ABSORPTION AND METABOLISM

A voluminous literature exists on the absorption and metabolism of vitamin B_{12} (cf Tracor Jitco, Inc. 1974). The subject also has recently been thoroughly reviewed by Matthews (1974).

Adams (1974) investigated the effect of food, saliva, dose of vitamin B_{12}, pentagastrin and hog intrinsic factor on vitamin B_{12} absorption in persons with pernicious anemia and subjects with normal vitamin B_{12} uptake. Vitamin absorption was measured by whole-body counting two weeks after oral administration of radiolabeled hydroxocobalamin or cyanocobalamin. In some cases a double isotope method was used to measure absorption. Vitamin B_{12} labeled with one cobalt radioisotope was given. Later, a second dose of vitamin B_{12} was given labeled with a second radioisotope; in addition, the substance being tested for its effect on vitamin B_{12} absorption was given at the same time. After appropriate background correction, the ratio between the uptake of each isotopically labeled species of the

vitamin could be determined to ascertain the effect of the substance. This method allows the subject to serve as his or her own internal control and eliminates spurious results due to inherent differences between individuals with respect to vitamin B_{12} uptake.

There was no significant difference in the percentage absorption of a 1 μg dose (0.017 mg per kg) of cyanocobalamin given to normal subjects when they were fasting or with a meal of toast and beans (Adams, 1974). There was a 37 percent increase in the percentage uptake of a 1 μg dose of cyanocobalamin by normal subjects if they were injected subcutaneously with 6 μg per kg of pentagastrin 30 minutes prior to cyanocobalamin dosing. Pentagastrin injection also increased the uptake of hydroxocobalamin but not to the same extent as for cyanocobalamin. Paradoxically, in persons with pernicious anemia, absorption of cyanocobalamin mediated by hog intrinsic factor was inhibited by prior administration of pentagastrin. The administration of increasing doses of hog intrinsic factor, up to 100 mg, caused an increase in the proportion of the 1 μg dose of cyanocobalamin absorbed by pernicious anemia patients. Doses of pentagastrin greater than 100 mg, up to 400 mg caused little additional cyanocobalamin absorption. When doses of 25 and 50 μg of cyanocobalamin were given to normal subjects, 3.5 and 4.1 percent were absorbed respectively; upon administration of 25 and 50 μg doses of hydroxocobalamin to normal subjects there was 2.2 and 2.1 percent absorption respectively. Lesser amounts of both compounds were absorbed when the same doses were given to pernicious anemia patients without any supplemental intrinsic factor.

Doscherholmen et al. (1974) investigated the effect of food on the absorption of vitamin B_{12} in man. Radiolabeled eggs were prepared by injecting a laying hen with ^{57}Co cyanocobalamin. The yolks were scrambled and fed to two normal, fasted volunteers. The meal also included toast and coffee. The portion of yolk fed each volunteer contained 1.12 μg of radiolabeled cyanocobalamin (0.019 μg per kg). Radioactivity appeared in the subjects' plasma after 3 or 4 hours and reached a peak 8 hours after ingestion of the meal; radioactivity gradually diminished in the following 160 hours. Doscherholmen et al. (1974) noted that the identical pattern was seen in persons consuming only labeled cyanocobalamin dissolved in water. It was not clear whether the latter results were obtained from the same persons who had ingested the radiolabeled egg yolk or whether Doscherholmen et al. (1974) referred to results obtained in other subjects.

Only 0.1 to 0.2 percent of a 0.4 to 6 μg oral dose of vitamin B_{12} was taken up in 2 hours by the liver and kidneys of male CFE rats whose stomachs had been ligated; if 1 percent of the nonionic surfactant, polyoxethylene-20-oleyl ether was present in the dosing solution, then from 0.7 to 2.3 percent of the vitamin B_{12} dose was absorbed from the ligated stomach

(Davis and Kreutler, 1971). Presence of the surfactant in the dosing solution also increased the uptake of vitamin B_{12} in intact animals.

Tennant et al. (1971) studied the absorption of a 0.02 μg (0.085 mg per kg) dose of ^{60}Co cyanocobalamin by three groups of germ-free Fisher rats. Absorption was studied after some animals had been inoculated with normal rat intestinal flora. The second group was monocontaminated with Escherichia coli while a third group remained germ free. There was no significant difference between the percentage of the dose absorbed by the three groups, although the amount remaining in the small intestine 8 hours after administration was significantly lower in the group inoculated with normal rat intestinal flora as compared to the other two groups. More than 50 percent of the ^{60}Co cyanocobalamin in the lumen of the cecum was bound in a readily sedimentable form in the E. coli inoculated and normal-flora inoculated rats compared to a value of less than 10 percent readily sedimentable ^{60}Co cyanocobalamin in germ-free animals. Thus, Tennant et al. (1971) postulated that binding of vitamin B_{12} by gut bacteria is not a significant factor in determining absorption of the vitamin.

Toskes et al. (1971) found a lowered urinary excretion of a 0.5 μg oral dose (0.0084 μg per kg) of radiolabeled cyanocobalamin in 9 of 22 patients with pancreatic exocrine insufficiency. The effect of a single dose of 12 g of hog pancreatic extract on the percentage urinary excretion of a 1 μg dose of cyanocobalamin was tested in four of the patients with pancreatic insufficiency showing decreased urinary excretion of radiolabeled cyanocobalamin. When the pancreatic extract was given in conjunction with the vitamin B_{12}, 18 percent of the dose was excreted in the urine, compared to only 4 percent excreted when the vitamin alone was given. Toskes et al. (1971) theorized that some factor in the pancreas may aid vitamin B_{12} absorption.

Vitamin B_{12} is not catabolized in man; the only loss occurs through biliary and urinary excretion. The bile may put out 3 to 7 μg of the substance daily, but most is reabsorbed. Daily urinary excretion is between 0 to 0.25 μg. From 50 to 90 percent of the body's store of vitamin B_{12} is in the liver (Herbert, 1975).

Vitamin B_{12} may play a role in the detoxification of cyanide in certain ethnic groups with specialized diets containing high amounts of cyanogenic substances; for example diets high in cassava (Anonymous, 1972).

II. FEEDING STUDIES

No long-term or short-term animal feeding studies with vitamin B_{12} were found for the time period considered for this review.

III. SPECIAL STUDIES

There are reports in the literature that persons may be sensitive to vitamin B_{12} (Tracor Jitco, 1974). Nalivko and Fedorovich (1974) described an allergic reaction to injected vitamin B_{12} in a 47-year-old woman welder. She was hospitalized for treatment of allergic dermatitis, possibly brought about by contact with metal dust and fumes to which she had been occupationally exposed for 12 years. The patient had positive patch tests for potassium bichromate, cobaltous chloride and nickel chloride. As part of her treatment regimen, she was injected with 200 µg of vitamin B_{12} (3.3 µg per kg). Forty minutes after injection of the compound the women exhibited headache, nausea, increased temperature, intense itching and blistering, and edema of subcutaneous tissue on the buttocks, back, abdomen, extremities, and breasts. The patient was treated for her allergic symptoms which subsequently disappeared in five days. The investigators suggested that her allergic response to vitamin B_{12} may have been due to cobalt which makes up 4.5 percent of vitamin B_{12}.

Eleven women and two men receiving vitamin B_{12} or vitamin B_{12} and vitamin B_6 developed acneiform exanthemata or had a worsening of pre-existing acne vulgaris (Braun-Falco and Lincke, 1976). The patients ranged in age from 13 to 67 years and received the vitamins either orally or by injection. The acne resolved upon discontinuation of the vitamins.

Gullberg (1973) detected a vitamin B_{12} binding protein in human breast milk. Vitamin B_{12} binding was measured by incubating milk with ^{57}Co cyanocobalamin and then separating protein-bound from free cyanocobalamin by passing the mixture through a gel filtration column and by measuring the radioactivity present in the protein fraction. The highest specific cyanocobalamin binding was found in unpasturized human milk; considerably less activity was seen in pooled pasturized human milk. Some binding was observed in fresh milk from one of five cows sampled; no vitamin B_{12} binding was observed in pasturized cows' milk or infant formula preparations based on cows' milk. Gullberg (1973) speculated that the vitamin B_{12} binding protein may compete with the intestinal flora for vitamin B_{12} and thereby regulate their growth since some E. coli strains require exogenous vitamin B_{12} or methionine (Davis and Mingioli, 1950).

IV. REFERENCES CITED

Adams, J.F., D.J. Clow, S.K. Ross, K. Boddy, P. King and M.A. Mahaffy. 1972. Factors affecting the absorption of vitamin B_{12}. Clin. Sci. 43:233-250.

Anonymous. 1972. Food and cyanide. Br. Med. J. (May 27):481-482.

Braun-Falco, O. and H. Lincke. 1976. Zur Frage der Vitamin B_6-/B_{12}-Akne: ein Beitrag zur Acne medicamentosa. Munch. Med. Wochenschr. 118(6):155-160.

Davis, B.D. and E.S. Mingioli. 1950. Mutants of Escherichia coli requiring methionine or vitamin B_{12}. J. Bact. 60: 17-28.

Davis, W.W. and C.J. Kreutler. 1971. Normal and promoted GI absorption of water-soluble substances. II. Absorption of vitamin B_{12} from ligated stomach and intact intestine of the rat. J. Pharm. Sci. 60:1651-1654.

Doscherholmen, A., J. McMahon and D. Ripley. 1974. Plasma absorption of vitamin B_{12} in food. J. Am. Med. Assoc. 227:800.

Gullberg, R. 1973. Possible influence of vitamin B_{12}-binding protein in milk on the intestinal flora in breast-fed infants. I. B_{12}-binding proteins in human and bovine milk. Scand. J. Gastroenterol. 8:497-503.

Herbert, V. 1975. Drugs effective in megaloblastic anemias: vitamin B_{12} and folic acid. Pages 1327-1338 and 1347-1349 in L.S. Goodman and A. Gilman, eds. The pharmacological basis of therapeutics, 5th ed. Macmillan Publishing Co., Inc., New York, N.Y.

Matthews, D.M. 1974. Absorption of water soluble vitamins. Biomembranes 46:847-915.

Nalivko, S.N. and S.V. Fedorovich. 1974. Allergicheskaya reaktsiya na vvedenie vitamina B_{12}. Vestn. Dermatol. Veneral. (8):66-67.

Tennant, B., M. Reina-Guerra and D. Harrold. 1971. Influence of micro-organisms on intestinal absorption. Ann. N.Y. Acad. Sci. 176:262-272.

Toskes, P.O., J. Hansell, J. Cerda and J.J. Deren. 1971. Vitamin B_{12} malabsorption in chronic pancreatic insufficiency: studies suggesting the presence of a pancreatic "intrinsic factor." N. Engl. J. Med. 284:627-632.

Tracor-Jitco, Inc. 1974. Monograph on vitamin B_{12}. Submitted under DHEW Contract No. FDA 72-104. Rockville, Md.